Abstract

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Abstract: *DESCRIPTION* (provided by applicant): Leukocyte integrins play key roles in vascular cell adhesion in host defense, inflammation, hemostasis, and metastasis. Our data suggest that the conformation of VLA-4 ("very late antigen"), the alpha4beta1 heteordimer, and its molecular extension (moving the ligand binding site away from the cell membrane) are associated with the affinity of VLA-4 for its ligand VCAM-1 ("vascular cell adhesion molecule"). An increase in affinity is accomplished through a decrease in molecular dissociation rate or increased residence time of the ligand. In turn, the extended conformation contributes to cell adhesion avidity (particularly the efficiency of cell adhesion). VLA-4 can be induced to change conformation and to extend by divalent cations and inside-out signal transduction (e.g. the pathway from a chemokine receptor to VLA-4) in conjunction with ligand binding. We have further suggested that the ability of VLA-4 to extend under the shear forces of cell adhesion promote extension of the molecule, increasing affinity and strength of cell-cell adhesion, and leading to intracellular signaling which further impacts the molecular conformation. At the current time, molecular probes for integrin function and conformation make use of both isosteric and allosteric regulators. The isosteric small molecules are targeted to the binding site of the native ligand. We have already developed a fluorescent peptide analogue for the ligand binding site of VLA-4. This high affinity ligand has been used with flow cytometry to measure VLA-4 affinity, its conformational state through fluorescence resonance energy transfer, and the time courses associated with their regulation. While allosteric regulators have been developed for other integrins, there are as yet no allosteric regulators for VLA-4. An allosteric regulator would not block the ligand binding site. Rather, distinct sets of allosteric molecules have the potential either to block the conformational and affinity changes of the integrin or to initiate them. Thus, the allosteric agonist is expected to cause integrin extension, increase affinity and increase avidity. It might promote intracellular signaling assocated with adhesion under shear. The antagonist of the allosteric site is expected to block extension, block affinity change, and block avidity change. It might also inhibit shear induced intracellular signaling. We have in hand several cell lines and physiological models with appropriate levels of integrin expression for high throughput screening for discovery of small molecule allosteric regulators by flow cytometry. These models provide the appropriate signaling and conformational characteristics to test all of the activities of the new small molecules that will be discovered. We have established a generic, homogeneous no-wash flow cytometric assay involving fluorescent peptides and stable cell lines expressing the desired receptor. We have validated that this assay can be performed in a high throughput model with VLA-4 and our fluorescent ligand with appropriate Z'.

Thesaurus Terms:
High throughput screening, MLSCN, Assay, Allosteric Ligands, VLA-4 Integrin, Leukocyte, vascular cell adhesion, alpha4beta1 heteordimer, VCAM-1, isosteric regulators, allosteric regulators, fluorescent peptide analogue, flow cytometry, fluorescence resonance energy transfer,

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